# A SYSTEMATIC STUDY OF THE ORGANISMS DISTRIBUTED UNDER THE NAME OF COCCOBACILLUS ACRIDIORUM D'HERELLE.

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A large amount of work has lately appeared dealing with d'Herelle's *Coccobacillus acridiorum* and his method of combating noxious grasshoppers. Some investigators have been able to confirm d'Herelle's results; others have been unable to do so, and since the entire subject seems to be in a state of confusion, I undertook a systematic study of a number of cultures which I obtained and which were distributed under the name of *Coccobacillus acridiorum* d'Herelle. As I suspected, some of the separate cultures proved to represent either different species or varieties of the same species. This fact may account for some of the contradictory views held by so many workers and it is my hope that this article will also demonstrate the need for attention to the ordinary principles of bacteriology which seem to be so persistently neglected by many entomologists.

In 1909 Dr. F. d'Herelle, while in the State of Yucatan, Mexico, noticed a heavy mortality in some flights of the destructive South American migratory locust *Schistocerca americana* Drury which arrived in the State from the borders of Guatemala. In 1911 the flights were all visited by this epizoötic and by 1912 it had reduced the number of locusts to such an extent that no invasion into Mexico occurred. In 1910 d'Herelle isolated a bacterium from the intestinal contents of cases of this disease. The organism was named by him *Coccobacillus acridiorum*. He was able to reproduce the disease and death by inoculating healthy grasshoppers with a culture of the Coccobacillus. These results were thought to be important by the Republic of Argentine which in 1911–12 requested d'Herelle to study the action of his bacterium against *Schistocerca paranensis* Burm, with a view towards its possible use in combating the pests. D'Herelle's methods apparently proved highly successful.

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D'Herelle's method consisted in obtaining a virulent form of his organism by twelve successive passages and then spraying dilutions of his cultures over sections of land infested by grasshoppers. The insects became infected by eating the contaminated food. The passages were performed by inoculation and the organism was considered to be virulent when death occurred in eight hours. The organism at its maximum virulence was supposed to cause death in three hours.

In 1913 Sergent and L'heritier tried out the efficacy of *Coccobacillus acridiorum* against *Stauronotus maroccanus* in Algeria. A general epidemic failed to develop in the field and the authors suggest that this failure may have been due to the presence of two autochthonous bacilli in the locusts which may have had an immunizing effect.

Lounsbury in 1913 attempted to combat *Zonocerus elegans* with d'Herelle's organism, in South Africa, but was unsuccessful.

Barber and Jones in 1913 performed field experiments with *Coccobacillus acridiorum* in the Philippines in an endeavor to check the injurious *Oedalens nigrofasciatus* De Geer and *Locusta migratoroides* R. and F. The experiments failed to show any satisfactory results.

During 1914–15 Béguet, Musso and Sergent conducted a campaign in Algeria against *Schistocerca peregrina* Oliv. These workers used d'Herelle's method in combination with the ordinary mechanical methods used for fighting the pests. It was found that d'Herelle's bacterium could not be used alone for the disease spreads too slowly. The combination of the two methods, however, proved helpful.

In 1915 Rorer reported that he had performed inoculation experiments with *Coccobacillus acridiorum* on *Schistocerca paranensis* and *Tropidacris dux* in Trinidad. He found that the organism was pathogenic to both insects and that the virulence could be increased by successive passages. Field experiments were not attempted.

Laines in 1915 reported that he was able to control grasshoppers in Honduras with d'Herelle's organism. A series of grasshoppers was inoculated from the abdominal substance of a series previously dead of the disease. By successive passages in this manner he claims to have obtained a high degree of virulence for the bacterium.

D'Herelle in 1915 controlled a severe outbreak of *Schistocerca peregrina* Oliv. in Tunisia by combining the use of his organism with the mechanical methods.

*Velu and Bouin in 1915* reported that d'Herelle's method gave encouraging results in combating *Schistocerca peregrina* in Morocco.

During September, 1915, Dr. L. O. Howard, U. S. Bureau of Entomology, received what was termed two sub-cultures of d'Herelle's Coccobacillus from Dr. Cicilio Lopez Ponce of Honduras. In a letter written by Dr. Ponce the latter says: "Under instructions from the Secretary of the Honduran Commission of Agriculture, who lives in Tegucigalpa, I have the honor of sending you by this post, in a registered package, two tubes of a culture of the *Coccobacillus acridiorum* d'Herrlle. Some time ago I came to this city from the neighboring Republic of Salvador with the object of taking charge of a laboratory devoted to the cultivation and propagation of this parasite, and I am pleased to inform you that the results could not have been more satisfactory."

From their experiments in Canada (1916) Du Porte and Vanderleck concluded that: "The results of our work indicate that d'Herelle's biological method for the control of locusts cannot take the place of the methods now in use under the conditions which obtain in eastern Canada. Should the disease become established, its spread would be extremely slow, owing to the non-migratory and non-cannibalistic habits of the native species. The ideal conditions for the effective use of this method are those such as d'Herelle and others found in South America and North Africa where the locusts were in quickly moving swarms and were markedly cannibalistic in their habits. Indeed, most of these writers have emphasized the fact that "acridiophagy" is the chief factor in the spread of the disease. Another hindrance to the effective use of this method lies in the presence of several native strains of a coccobacillus identical with or closely related to d'Herelle's. These organisms are undoubtedly responsible for the immunity of the locusts to a mild infection of *Coccobacillus acridiorum*."

A perusal of the literature of the subject shows that five out of nine articles report encouraging field results by the use of d'Herelle's *Coccobacillus acridiorum*. These five reports all deal with the genus *Schistocerca* represented by the species *americana*, *paranensis*, and *peregrina*. The unsuccessful reports deal with a variety of grasshopper genera such as *Stauronotus*, *Zonocerus*, *Oedalens*, *Locusta* and *Melanoplus*. The bacterium may be very effective when used against certain species of the

genus *Schistocerca*, whereas it may be impossible to establish an epidemic in the field amongst members of certain other genera. All of the workers reported that the bacteria they used were pathogenic in their laboratory experiments. The field failures may be due to differences between the habits of the members of the genus *Schistocerca* and those of other genera. Differences between the climates of the separate countries where *Coccobacillus acridiorum* was used may also account for the varied results. The natural immunity of different genera or species is another factor worthy of consideration. Shall we therefore, owing to several failures, condemn d'Herelle's method under certain conditions? Obviously not.

D'Herelle and other workers who used his organism and methods successfully consider the following requirements the most necessary to the rapid spread of the disease in the field.

1. Cannibalistic habits of the insects (as very frequently exhibited by the genus *Schistocerca*).
2. Migratory habits (exhibited by the genus *Schistocerca*).
3. Dense hopper infestation.
4. Absence in hoppers of bacteria closely related to the *Coccobacillus acridiorum*. The presence of such organisms may have an immunizing effect.
5. Not an overabundance of normal food. When food becomes scarce due to the hopper infestation, the insects acquire cannibalistic habits which are favorable to the spread of the disease.
6. High temperature. The disease spreads more rapidly at the optimum temperature.
7. Absence of excessive rain. A heavy rain paralyzes the march of the epidemic.

All of the foregoing factors are undoubtedly highly important, but the writer should like to add one more requirement absolutely necessary for the study of this subject, namely:

8. The use of the same organism by the different investigators. Carefully controlled cultures should be distributed and used. The cultural and especially the bio-chemical characters of *Coccobacillus acridiorum* should be referred to constantly. Reference to morphological characters solely, as has been done so often, is worthless.

During December, 1915, I received from Dr. L. O. Howard, Chief of the U. S. Bureau of Entomology, two nutrient agar tubes containing pure cultures of a bacterium. These cultures were forwarded to Washington at the request of Dr. Howard by Dr. Cicilio Lopez Ponce, representing the Secretary of the Honduran Agricultural Commission of Tegucigalpa, Honduras.

The cultures were supposed to represent d'Herelle's *Coccobacillus acridiorum* and Dr. Ponce claimed to have obtained striking results with them in his field experiments.

During February, 1917, I received two cultures of the supposed *Coccobacillus acridiorum* direct from Dr. F. d'Herelle who is now at the Pasteur Institute, Paris. One culture was labeled "Souche Cham" which d'Herelle informed me is identical with the one I received from Dr. Ponce of Honduras. The other culture was labeled "Souche Sidi" and according to d'Herelle represented a strain of *Coccobacillus acridiorum* passed through a series of grasshoppers in Tunisia in 1915.

Also in February, 1917, I received through the kindness of Dr. C. Gordon Hewitt a pure culture of the supposed *Coccobacillus acridiorum* from Messrs. Du Porte and Vanderleck, who have performed some interesting experiments with this bacterium in eastern Canada. Dr. Hewitt, in a letter to me, stated that he received this culture direct from the Pasteur Institute in Paris.

I made a careful systematic study of these four cultures, compared them with one another as well as with the published descriptions of d'Herelle, and those of Du Porte and Vanderleck. The cultures differ from one another more or less. A table on page 25 shows the most salient differences and similarities. Since the bacterium sent by Ponce from Honduras seems to be an organism new to bacteriological literature, I have described it as a new species and named it *Bacillus poncei* in honor of Dr. Cicilio Lopez Ponce. I have also redescribed the three other cultures.*

*Bacillus poncei* is certainly not a Coccobacillus. It is a true bacillus, not in the least pleomorphic, no matter on what media it is grown. In this respect it certainly differs from d'Herelle's description. The latter emphatically states that his organism is highly pleomorphic, all stages between bacilli and cocci being observed in the same pure culture. *Bacillus poncei* produces much acid in milk; *Coccobacillus acridiorum*, according to d'Herelle, strong alkalinity. In so far as the production of ammonia and the fermentation (with gas) of dextrose, levulose and maltose are concerned the two organisms agree. D'Herelle's cultural and bio-chemical descriptions are so meagre that it is difficult to ascertain his exact meaning.

* The detailed descriptions will be found appended to this article.

Culture "Souche Cham" is highly pleomorphic. Milk is rendered acid, but is not coagulated. D'Herelle's organism should render milk alkaline and coagulate it. In the production of ammonia and in the fermentation (with gas) of dextrose, levulose and maltose, the two organisms agree. As can be seen from the table on page 25 "Souche Cham" differs greatly from *B. poncei*. Strange as it may seem, "Souche Sidi" and "Souche Cham," the two cultures sent by d'Herelle himself differ from one another. "Souche Sidi" is slightly pleomorphic, but this character is not nearly so pronounced as is the case with "Souche Cham." "Souche Sidi" coagulates milk, whereas "Souche Cham" does not. "Souche Sidi" reduces litmus milk; "Souche Cham" does not. "Souche Sidi" does not ferment (with gas) lactose and adonit; "Souche Cham" ferments both of these carbohydrates with the fermentation of gas (Hydrogen $+CO_2$). "Souche Sidi" does not tally with d'Herelle's description nor with *B. poncei*.

Du Porte and Vanderleck's culture agrees with the culture I received from d'Herelle under the name of "Souche Sidi." Curiously enough, however, my description of Du Porte and Vanderleck's culture does not entirely agree with the description given by these writers. I agree with them in so far as the morphological characters are concerned. My gelatin stabs, however, showed liquifaction after about eight weeks; they claim that gelatin is not liquified. My litmus was reduced; Du Porte and Vanderleck claim "no reduction." I am unable accurately to interpret the results of Du Porte and Vanderleck's carbohydrate fermentation tests for the reason that they do not state whether fermentation was accompanied by the formation of gas and acid or merely acid alone. I assume they mean gas formation, in which case, as will be seen from the table on page 25, our lactose tests differ.

What can one conclude from these results? Only this, namely, that different organisms are being distributed under the name of *Coccobacillus acridiorum*. I should, moreover, like to suggest that d'Herelle redescribe the organism concerned in his grasshopper epidemic more accurately so that other workers may know to which bacterium reference is made. Judging from the morphological descriptions alone I think d'Herelle has reference to the highly pleomorphic organism which he sent me labeled "Souche Cham," but of course, this is merely a conjecture. Du Porte and Vanderleck found several pleomorphic organisms native to grassphopers in eastern Canada.

TABLE SHOWING MOST STRIKING DIFFERENCES AND SIMILARITIES BETWEEN CULTURES DISTRIBUTED UNDER THE NAME COCCOBACILLUS ACRIDIORUM D'HERELLE.

| | MORPHOLOGY | GELATIN STAB | MILK | LITMUS MILK | $NH_3$ on POTATO | NITRATE REDUCTION | INDOL | DEXTROSE | LEVULOSE | SACCHAROSE | MALTOSE | LACTOSE | MANNIT | ADONIT | DULCIT |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Bacillus poncei from Honduras | Bacilli | No liquifaction | Strong acidity. Coagulation. | Strong acidity Coagulation Reduction | + | + | O | + | + | O | + | + | + | + | O |
| "Souche Sidi" from d'Herelle | Bacilli and coccoid forms | Liquifaction | Weak acidity. Coagulation. | Weak acidity Coagulation Reduction | + | + | O | + | + | + | + | O | + | O | O |
| Culture sent by Du Porte & Vanderleck | Bacilli and coccoid forms | Liquifaction | Weak acidity. Coagulation. | Weak acidity Coagulation Reduction | + | + | O | + | + | + | + | O | + | O | O |
| "Souche Cham" from d'Herelle | All stages between bacilli and diplococci | Gas. Liquifaction | Weak acidity. No coagulation. | Weak acidity No coagulation No reduction | + | + | O | + | + | + | + | + | + | + | O |
| d'Herelle's Original Description | All stages between bacilli and cocci | Not mentioned | Strong alkalinity. Coagulation. | Not mentioned | + | Not men-tion'd | Not men-tion'd | + | + | Not men-tion'd | + | Not men-tion'd | Not men-tion'd | Not men-tion'd | Not men-tion'd |
| Du Porte & Vanderleck's Description | Bacilli and diplococci | No liquifaction | Acidity. Coagulation. | Weak acidity Coagulation No reduction | Not men-tion'd | Not men-tion'd | O | + | Not men-tion'd | + | Not men-tion'd | + | Not men-tion'd | O | O |

EXPERIMENTATION WITH CULTURES.

In order to test the pathogenicity of the separate cultures laboratory experiments were performed with *Bacillus poncei* and with d'Herelle's cultures labeled "Souche Cham" and "Souche Sidi." I attempted no experiments with the culture obtained from Messrs. Du Porte and Vanderleck for the reason that my systematic study showed this culture to be identical with d'Herelle's "Souche Sidi" strain.

In all of my experiments the most painstaking bacteriological technicalities were observed, so I shall not undertake to describe all of the tiresome and well known methods in vogue such as using sterile instruments, etc., for injecting and operating upon a grasshopper. Suffice it to say, that sterile containers in the form of battery jars were found extremely useful in performing my experiments. Glass plates covered the jars in order to keep the hoppers from jumping out. These plates had the further advantage of keeping the corn leaves, with which we fed the insects, fresh. Prior to the injection the hoppers were always washed with 95% alcohol. This must not be used too freely, otherwise, the grasshoppers may die and after its use one must wait a minute or so for the alcohol to evaporate before injecting. A small amount of alcohol entering the wound, however minute, caused by the hypodermic needle usually ends fatally. The inoculations were always performed between the metathorax and the first abdominal segment on the ventral side. In order to avoid rupturing the gut or otherwise injuring the insect, two operators are absolutely necessary to perform successful inoculations. One person must carefully, but firmly hold the insect while the other inoculates. I performed a number of tests in order to determine whether my technical precautions were sufficient and I found them satisfactory. For example: I washed off a large series of grasshoppers with alcohol and then injected with sterile water. Some of the insects I permitted to live until they seemed to die of natural causes; others I killed after periods of one and two weeks in order to inoculate culture tubes with some blood obtained by bathing the trochanter and femur with alcohol and then breaking the joint by a swift movement. The culture tubes remained perfectly sterile.

*Experiments with Bacillus poncei.*

On reviewing the tables which illustrate my experiments with *B. poncei* and the other bacteria investigators in this subject may wonder why I used females more often in preference to males. The reasons are these: Female grasshoppers are much larger than the males and consequently easier to handle. Moreover, they seem to be hardier and withstand the alcohol bath and hypodermic needle much better than the males. Finally, the females naturally live longer which is, of course, a decided advantage in any experiment.

Another inconsistency in my experiments seems evident from the fact that at times I used a smaller or a larger number of animals in one experiment than in another. This was found necessary for the reason that large numbers of female grasshoppers of the desired species, sufficiently mature for experimentation, were sometimes difficult to find in the region where my laboratory experiments were performed.

The tables given on pages 38–41 are self-explanatory. The insects were always inoculated with one drop (1/10 c. c.) of the particular fluid. The emulsion of the six months old agar culture of *B. poncei* given in Table I was prepared by adding 10 c. c. of sterile water to the old culture and shaking the tube vigorously. The emulsions of the intestinal contents of dead animals were prepared by dissecting out the intestines under aseptic conditions and triturating in sterile test tubes containing 5 c. c. of sterile water. This material, owing to the fact that it contained shreds of tissue was filtered in a sterile filter especially prepared for the experiments and from which unfiltered air was excluded.

Tables II, III and IV represent passage infections modeled after the experiments performed by other workers. By an examination of Tables I–IV it would seem that I had increased the virulence of *B. poncei*, while the deaths represented on Tables I and II extend over a long period of time; at the second and especially at the third passage Tables III and IV, the number of days elapsing between infection and death are considerably shortened.

The optimist would at once proclaim this as evidence for increase in virulence, but such is not the case. The three animals dead in the last experiment were carefully examined

and an earnest attempt was made to recover *B. poncei*, but I utterly failed. I inoculated a variety of media from the blood, from various tissues and from the intestines. I plated from these media and tested all suspicious looking colonies on the required media, (media given in my descriptions of organism) in the sugar tubes and performed the nitrate and indol tests as well but without success. About a half-dozen other organisms were found, but *B. poncei* failed to reveal itself. What killed the grasshoppers? The five deaths represented on Table I were probably due to *B. poncei*, but the deaths in the three passage infections were due to the careless way in which these experiments were carried out. Since this method has been used by practically all workers on this subject, I wish to point out its absolute worthlessness. Grasshopper intestines, as a large number of observations convinced me, are not only often full of gregarines and flagellates, but contain many species of bacteria (intestinal flora). By performing such passage infections as outlined in my tables one simply inoculates the animals with an indefinite quantity of intestinal flora. No wonder the animals succumbed. What then became of *B. poncei?* This bacterium was either destroyed by the countless other introduced bacteria or was killed by the grasshopper blood cells (phagocytocis) or other immunity principles. If the grasshoppers are to be inoculated in the body cavity why should so many investigators choose the intestines for further passages? Why not perform the passages with the blood? Of course, a sufficient number of the organisms introduced should cling to the outside of the intestines when these are removed, but other organisms within the intestines are likewise introduced. I also failed to obtain pure cultures of *B. poncei* by resort to blood passages on the animals I used (*Melanoplus femur-rubrum* and *Encoptolophus sordidus*) for the reason that the blood seems to act antagonistically towards the bacterium in question and destroys it in most cases. Nevertheless, other organisms are carried along since the toxins or other products introduced cause a disturbance of some sort which in turn causes the gut of the grasshopper to rupture liberating the intestinal flora into the body cavity.

Tables V, VI and VII represent another series of experiments performed along the same lines as the preceding. The results were exactly similar. I know of no way in which passage

infections can be performed in this manner. Tables VIII, IX and X represent another series of passages performed on another species of grasshopper, *Encoptolophus sordidus*. Even after the 1st passage I failed to recover *B. poncei*. Strange as it may appear, I recovered *B. poncei* from one animal dead in the 2nd passage.

Table XI represents thirty-five animals (*M. femur-rubrum*) inoculated with a twenty-four hour bouillon culture of *B. poncei*. The organism in question was recovered only three times.

Table XII demonstrates what is meant by the rupture of the gut after a foreign toxin or protein is introduced into the blood. In order to see whether I was rupturing the intestines myself by introducing the hypodermic needle, I injected a large series of grasshoppers with a dead culture of *B. poncei*. After three days I inoculated some bouillon tubes with some of the blood taken from these animals. The tubes remained perfectly sterile.

Tables XIII and XIV represent experiments on infection by feeding. Here the organisms were introduced into the alimentary tract. If *B. poncei* is pathogenic at all, I thought, this would be the most natural method of infection. I failed, however, to recover the organism either from the feces, from the living infected animals, or from the alimentary tract of the dead. From what did these animals die? Possibly from endotoxins liberated from *B. poncei*, which was destroyed within the grasshopper stomach and intestines.

The method of spraying the culture on the food foliage consisted in diluting the culture one-half with sterile water and spraying with a fine atomizer until the leaves were visibly wet.

### *Conclusions on Experiments with B. poncei.*

I conclude from the foregoing experiments that *B. poncei* is pathogenic to *Melanoplus femur-rubrum* and *Encoptolophus sordidus*. In most cases, however, I failed to recover the organism from the blood, the alimentary tract and from the feces. My experiments lead me to believe that insects can develop immunity principles which can more or less successfully cope with certain foreign organisms. The following experiment will further assist in substantiating this view. October 12, 1916, I inoculated six female *M. femur-rubrum* with a twenty-four

hour bouillon culture of *B. poncei*. October 13th the animals were all alive. I pulled out one metathoracic leg from each animal and permitted a drop of blood from each to flow into a nutrient bouillon tube. Three tubes were kept at room temperature and three were incubated, yet all six remained perfectly sterile. Stained smears of some of the blood also failed to reveal any micro-organisms. Sooner or later, I think, the gut would have ruptured liberating the intestinal flora into the body cavity, so I thought it best to make the tests the second day.

I further conclude that passage infections performed by using the alimentary tract are hopeless on account of the extensive flora. Blood passages, with *B. poncei*, were likewise useless, in most cases, for the reason that the gut ruptured after a short time. Passages by means of the blood are possible with other bacteria, however, as I will show later.

### *Experiments with Cultures "Souche Cham" and "Souche Sidi."*

The infection experiments with "Souche Cham" and "Souche Sidi" were much more satisfactory than those with *B. poncei*. In regard to "Souche Cham," I successfully performed two passages, but curiously enough, as can be seen from Tables XV–XVII (1 and 2), obtained no increase in virulence. Perhaps if I had measured the time between inoculation and death in hours instead of in days, I might have noticed something, but many deaths unfortunately occurred during the night. However, measurement of time in days is sufficient and if a marked increase in virulence had manifested itself, I surely would have noticed it.

The gut of *M. atlanis* never ruptured, so the blood or muscle tissue could readily be used as a basis for further inoculations. In no case, however, can extracts from the stomach or the intestines be used for further passages. A series of examinations conclusively proved that these are invariably contaminated even in perfectly normal looking animals.

Tables XVIII and XIX represent experiments dealing with food infections. *M. atlanis* was also the subject for these tests. In general the time between infection and death is somewhat extended which is to be expected in this mode of experiment,

still it seems to me that the organism acts very quickly. These "per os" infections really mean more than the inoculation experiments for the reason that it is the natural way in which the bacterium would invade the insect. Of course, laboratory passages, where pure recoveries are required, are impossible to perform by this method of infection unless one plated between each infection. Since my experiments showed the futility of passages, in so far as increase in virulence is concerned, I did not see any advantage in doing an extra amount of tedious work. It seems to me that the organism is sufficiently virulent even in old cultures, so that if one could succeed in establishing a center of infection in the field an epidemic would soon follow provided certain conditions were favorable.

Tables XX and XXI also represent food infections. *M. bivittatus* was the subject. If it is permissible to judge from two experiments the organism does not seem to be so pathogenic to this insect. A number of insects in the XX experiment succumbed to parasitism by *Mermis ferruginea*, a nematode.

Table XXII represents the same sort of an experiment as the preceding with the exception that *M. femur-rubrum* was the subject. "Souche Cham" also does not seem to be as highly pathogenic to this species as it is to *M. atlanis*.

Table XXIII represents an inoculation experiment with "Souche Sidi." *M. atlanis* was the subject. The period from infection to death extends over a period of six days. This seems to show that "Souche Sidi" is not as pathogenic as "Souche Cham."

Table XXIV represents a food infection experiment with the same culture and the same subject. The period from infection to death is also, in general, prolonged. Two animals died naturally although I am certain they became infected.

In all of the food infection experiments the grasshoppers were given barely enough leaves in order to insure their eating everything in 12–24 hours.

Table XXV represents a food infection experiment with "Souche Sidi." The subject in this case was *M. bivittatus*. The pathogenicity of "Souche Sidi" for this species seems to be the same as for *M. atlanis*.

Suitable checks accompanied all of my experiments. These always died of old age or of *Mermis* parasitism, but seemed not to suffer naturally from any endemic disease. At times I found some checks prematurely dead, but I traced this to $CO_2$ asphyxiation and on replacing my glass plates, which covered the battery jar containers, with cheese-cloth tops, I overcame this difficulty. The glass plates are splendid, however, unless one confines too many insects in one jar.

As can be seen on examining the tables, I performed a large series of post mortem tests. This means that stained smears were studied and that the material was "plated out," colonies isolated and the species studied on different media, and their bio-chemical characters in carbohydrates, etc., observed. Of course, some of these final tests were finished long after the conclusion of the grasshopper season. It is absolutely impossible to perform in a short time, the huge amount of work which experiments of this sort require.

*Conclusions on experiments with cultures "Souche Cham" and "Souche Sidi."*

1. "Souche Cham" is pathogenic to *M. atlanis*, *M. bivittatus* and *M. femur-rubrum*.

2. "Souche Cham" is not as pathogenic to *M. bivittatus* and *M. femur-rubrum* as to *M. atlanis*.

3. Passage infections with "Souche Cham" were possible, but no increase in virulence was observed.

4. The gut of *M. atlanis* does not rupture, and for this reason the blood and muscle tissue can be used for passage infections.

5. Extracts from the stomach or intestines can not be used for passage infections.

6. In food infections the time between inoculation and death is somewhat extended.

7. "Souche Cham" and "Souche Sidi" are quite virulent even in old cultures.

8. "Souche Sidi" is not as pathogenic to *M. atlanis* and *M. bivittatus* as "Souche Cham."

9. No passage infections with "Souche Sidi" were attempted.

FIELD EXPERIMENTS.

*Melanoplus atlanis* is a serious pest in certain regions of the State of Vermont. Since this species occurs in dense swarms and since it acquires cannibalistic habits when natural food becomes scarce, I thought it would be splendid material for field work. Mr. A. M. Wilcox and I have instituted a large series of field experiments with cultures "Souche Cham" and "Souche Sidi" in Vermont, but we wish to await the passage of at least another season before drawing any conclusions. The hurried method of rushing into print field observations dealing with a single season's work is deplorable. The amount of work which is necessary before coming to any conclusions at all is so immense that an army of trained workers co-operating in every possible way could not obtain final results after a single season's work.

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Culture sent by Dr. Cicilio Lopez Ponce of Honduras under the name of *Coccobacillus acridiorum:*

*Bacillus poncei sp. nov.*

*Morphology.* From 1½% nutrient agar stroke 24 hours old, long rods. From 1½% potato agar stroke 24 hours old, long rods and some short rods. From milk 48 hours old, many short rods. Average length 2.2μ. Average width .9μ. Motile. Gram negative. Stains readily.

*Nutrient agar stroke.* 1½%. Neutral. Growth moderate, spreading, flat, glistening, smooth, white, opaque, odor absent, butyrous, medium unchanged.

*Potato agar stroke.* 1½%. Neutral. Growth very luxuriant, arborescent, flat, glistening, smooth, white, opaque, odor absent, butyrous, medium unchanged.

*Potato.* Growth abundant, spreading, flat, glistening, smooth, white, odor absent, butyrous, medium unchanged.

*Gelatin stab.* Growth best at top, beaded, no liquifaction, medium unchanged.

*Nutrient broth.* Neutral. Pellicle, clouding strong, no clearing after 15 days, odor absent, slight sediment.

*Milk.* Acid. Coagulation in six days. Extrusion of whey in six to ten days, no peptonization, color of medium unchanged.

*Litmus milk.* Acid, coagulation, prompt reduction.

*Gelatin colonies.* Growth slow, white, round, slightly raised, edge entire, no liquifaction.

*Agar colonies.* Growth slow, white, round, smooth, raised slightly, edge entire, amorphous, diameter 4 mm.

*Ammonia production.* Feeble.

*Nitrate solution.* Nitrates reduced to nitrites.
*Indol production.* Absent.
*Hydrogen sulphide production.* Absent.
*Fermentation of carbohydrates with gas.*

| | | | |
|---|---|---|---|
| Dextrose | + | Lactose | + |
| Levulose | + | Mannit | + |
| Saccharose | O | Adonit | + |
| Maltose | + | Dulcit | O |

*Pathogenicity.* Pathogenic to *Melanoplus femur-rubrum*, *Encoptolophus sordidus*, and *Gryllus pennsylvanicus*. Pathogenicity not tested out on any other forms.

Culture sent by d'Herelle under the name *Coccobacillus acridiorum*. Culture labeled "Souche Cham":

*Morphology.* From 1½% nutrient agar stroke 48 hours old, small diplococci. All very uniform. No bacilli. In water of condensation all transition forms between diplococci and bacilli. Highly polymorphous.* From milk 48 hours old, small diplococci, no bacilli. From bouillon 48 hours old, all intermediate stages between true bacilli and coccus forms. Nutrient bouillon is a favorable medium for the development of the bacillus forms. Solid media like nutrient and potato agar are favorable for the development of the diplococcus forms. This can be easily demonstrated by transferring from the liquid to the solid medium and vice versa. Diameter of cocci .6$\mu$. Length of bacilli .7-1.5$\mu$. Motile. Gram negative. Stains readily.

*Nutrient agar stroke.* 1½%. Neutral. Growth abundant, spreading, flat, glistening, smooth, opaque, odor absent, butyrous, color of medium unchanged.

*Potato agar stroke.* 1½%. Neutral. Growth very luxuriant, spreading, flat, glistening, smooth, opaque, odor absent, butyrous, color of medium unchanged.

*Potato.* Growth abundant, whitish.

*Gelatin stab.* Growth uniform, beaded, gas, liquifaction, medium unchanged.

*Nutrient broth.* Ring, slight pellicle, clouding strong, sediment abundant, odor absent, no clearing after fifteen days.

*Milk.* Weak acidity, no coagulation, color of medium unchanged.

*Litmus milk.* Weak acidity, no coagulation, no reduction.

*Gelatin colonies.* Growth rapid, round, convex, edge entire, gas, diameter of colony .5-1 mm.

*Nutrient agar colonies.* Growth rapid, round, smooth, flat, edge entire, amorphous, diameter of colony 2.5-3 mm.

*Ammonia production.* Positive.

*Nitrate solution.* Nitrates reduced to nitrites.

*Indol production.* Negative.

*For an interesting article on pleomorphism see: Studies in pleomorphism in Typhus and other diseases by Edward C. Hort. Abstract in Jour. Royal Micros. Soc., December, 1916.

*Hydrogen sulphide production.* Negative.

*Fermentation of carbohydrates with gas.*

| | | | |
|---|---|---|---|
| Dextrose | + | Lactose | + |
| Levulose | + | Mannit | + |
| Saccharose | + | Adonit | + |
| Maltose | + | Dulcit | O |

*Pathogenicity.* Pathogenic to *Melanoplus atlanis*, *Melanoplus bivittatus*, and *Melanoplus femur-rubrum*. Pathogenicity not tested out on any other forms.

Culture sent by d'Herelle under the name *Coccobacillus acridiorum*. Culture labeled "Souche Sidi":

*Morphology.* From 1½% nutrient agar stroke 48 hours old, short bacilli dominant forms; some coccoid forms. In water of condensation bacillus forms typical. From milk 48 hours old, bacillus forms dominant; some coccoid forms. From bouillon 48 hours old, typical bacillus forms dominant; few coccoid forms. Not as polymorphous as culture "Souche Cham." Length of bacilli .8-1.5μ. Motile. Gram negative. Stains readily.

*Nutrient agar stroke.* 1½%. Neutral. Growth abundant, spreading, flat, glistening, smooth, opaque, odor absent, butyrous, color of medium unchanged.

*Potato agar stroke.* 1½%. Neutral. Growth very luxuriant, spreading, flat, glistening, smooth, opaque, odor absent, butyrous, color of medium unchanged.

*Potato.* Growth abundant, whitish.

*Gelatin stab.* Growth uniform, beaded, liquifaction, medium unchanged.

*Nutrient broth.* Ring, slight pellicle, clouding strong, sediment abundant, odor absent, no clearing after fifteen days.

*Milk.* Weak acidity, coagulation delayed, extrusion of whey, no peptonization, color of medium unchanged.

*Litmus milk.* Weak acidity, coagulation, extrusion of whey, reduction complete.

*Gelatin colonies.* Growth rapid, round, convex, edge entire, no liquifaction, diameter of colony .5-1 mm.

*Nutrient agar colonies.* Growth rapid, round, smooth, flat, edge entire, coarsely granular, diameter of colony 2.5-3 mm.

*Ammonia production.* Positive.

*Nitrate solution.* Nitrates reduced to nitrites.

*Indol production.* Negative.

*Hydrogen sulphide production.* Negative.

*Fermentation of carbohydrates with gas.*

| | | | |
|---|---|---|---|
| Dextrose | + | Lactose | O |
| Levulose | + | Mannit | + |
| Saccharose | + | Adonit | O |
| Maltose | + | Dulcit | O |

*Pathogenicity.* Pathogenic to *Melanoplus atlanis* and *Melanoplus bivittatus*. Pathogenicity not tested out on any other forms.

Culture sent by Messrs. Du Porte and Vanderleck of Canada, who received same from d'Herelle under the name of *Coccobacillus acridiorum:*

*Morphology.* From 1½% nutrient agar stroke 48 hours old, short bacilli dominant forms; some coccoid forms. In water of condensation bacillus forms typical. From milk 48 hours old, bacillus forms dominant; some coccoid forms. From bouillon 48 hours old, typical bacillus forms abundant; few coccoid forms. Not as polymorphus as culture "Souche Cham." Length of bacilli .8-1.5$\mu$. Motile. Gram negative. Stains readily.

*Nutrient agar stroke.* 1½%. Neutral. Growth abundant, spreading, flat, glistening, smooth, opaque, odor absent, butyrous, medium unchanged.

*Potato agar stroke.* 1½%. Neutral. Growth very luxuriant, spreading, flat, glistening, smooth, opaque, odor absent, butyrous, medium unchanged.

*Potato.* Growth abundant, whitish.

*Gelatin stab.* Growth uniform, beaded, liquifaction, medium unchanged.

*Nutrient broth.* Ring, slight pellicle, clouding strong, sediment abundant, odor absent, no clearing after fifteen days.

*Milk.* Weak acidity, coagulation delayed, extrusion of whey, no peptonization, color of medium unchanged.

*Litmus milk.* Weak acidity, coagulation, extrusion of whey, reduction complete.

*Gelatin colonies.* Growth rapid, round, convex, edge entire, no liquifaction, diameter of colony .5-1 mm.

*Nutrient agar colonies.* Growth rapid, round, smooth, flat, edge entire, coarsely granular, diameter of colony 2.5-3 mm.

*Ammonia production.* Positive.

*Nitrate solution.* Nitrates reduced to nitrites.

*Indol production.* Negative.

*Hydrogen sulphide production.* Negative.

*Fermentation of carbohydrates with gas.*

| | | | |
|---|---|---|---|
| Dextrose | + | Lactose | O |
| Levulose | + | Mannit | + |
| Saccharose | + | Dulcit | O |
| Maltose | + | Adonit | O |

*Pathogenicity.* Pathogenic to *Melanoplus atlanis* and *Melanoplus bivittatus.* Pathogenicity not tested out on any other forms.

Original description by d'Herelle of *Coccobacillus acridiorum:*

*Morphology.* Media from which the morphological observations were made not mentioned. Short bacillus, slightly oval, very polymorphous. Cocci .6$\mu$, bacilli .4-.6$\mu$ by .9-1.5$\mu$. Motile, peripheral flagellæ. Gram negative, stains readily.

*Potato.* Growth creamy. Water of condensation sirupy, reaction strongly alkaline.

*Gelatin.* Not liquified.

*Nutrient broth.* Development rapid at 37°. Clouding from fourth hour on. After several days a very light veil appears. Bouillon clears after three weeks, producing a sediment. A young culture agitated produces silky waves. Odor of Liebig's extract. Rendered strongly alkaline.

*Milk.* Coagulated and rendered strongly alkaline.

*Nutrient agar colonies.* Visible after 12 hours. After 18 hours they are 2–3 mm. in diameter. Circular, waxy. Below surface spherical, whitish, opaque.

*Fermentation of carbohydrates.*

| | | |
|---|---|---|
| Dextrose | + | Organism renders medium containing one |
| Levulose | + | of these sugars slightly acid than |
| Maltose | + | alkaline. |
| Galactose | + | |

*Oxygen requirements.* Facultative anaerobe.

*Pathogenicity.* Pathogenic to various Acrididæ, ants and caterpillars.

Description by Du Porte and Vanderleck of culture sent to them from d'Herelle under the name *Coccobacillus acridiorum:*

*Morphology.* From agar slope 20 hours old, short rods or cocci, some oval, polymorphous. 0.7–1.0$\mu$. In milk culture they appear often as diplococci. Motile. Gram negative. Amylgram positive. Stain readily.

*Agar stroke.* Abundant growth, spreading, flat, glistening, smooth, dirty white to bluish white, opaque, butyrous, medium unchanged. On 1% agar the cultures are arborescent and transparent.

*Potato.* Abundant growth, spreading, flat, glistening, smooth, butyrous; color from dirty white to yellow.

*Gelatin stab.* Uniform growth, line of puncture filiform. No liquifaction, medium unchanged. Stab brownish yellow.

*Nutrient broth.* Pellicle or ring, turbidity, slight sediment, no clearing after 14 days, odor of beef extract.

*Milk.* At first gas production without coagulation. Delayed coagulation in 2–8 days, acid reaction after 8 days, no peptonization, medium unchanged, no extrusion of whey.

*Litmus milk.* Gas production, weak acidity, no reduction. After four days partial to complete coagulation, acid.

*Gelatin colonies.* Growth slow, round, raised, edge entire, yellow. Three weeks, 2 mm. in diameter, yellow white. No liquifaction.

*Agar colonies.* Rapid growth, irregular, round, smooth, flat, edge entire, amorphous, dirty white to blue, transparent. Growth more restricted on 1½% than on 1% agar.

*Aesculin bilesalt agar.* Weak field after 24 to 48 hours.

*Neutral red bilesalt agar.* Strong fluorescence, red spreading.

*Indol production.* Negative.

*Fermentation of carbohydrates.*

| | | | |
|---|---|---|---|
| Dextrose | + | Rafinose | + |
| Saccharose | + | Arabinose | + |
| Lactose | + (weak) | Adonit | O |
| Galactose | + | Dulcit | O |
| Muscle sugar | + | | |

*Pathogenicity.* Pathogenic to locusts and grasshoppers. Injection fatal within 24 hours.

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TABLE I.

Ten animals injected with an emulsion of a 6 months old agar culture of *B. poncei.* ♀ ♀ *M. femur-rubrum* used.

| No. of days.... | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| No. of deaths.. | 1 | | | | 1 | | | 1 | | | | | 1 | 1 | 5 lived |

TABLE II.

Eight animals injected with an emulsion of intestinal contents of animal dead on 8th day. ♀ ♀ *M. femur-rubrum* used. 1st passage.

| No. of days..... | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| No. of deaths..... | | 1 | | | | 2 | 1 | 2 | | 1 | 1 lived |

TABLE III.

Three animals injected with an emulsion of intestinal contents of an animal dead on the 6th day. ♀ ♀ *M. femur-rubrum* used. 2nd passage.

| No. of days..... | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| No. of deaths..... | | | 1 | 1 | 1 |

TABLE IV.

Three animals injected with an emulsion of intestinal contents of last one dead in previous experiment. ♀ ♀ *M. femur-rubrum* used. 3rd passage.

| No. of days..... | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| No. of deaths..... | | 3 | | |

The three animals dead on 2nd day were "tested." *B. poncei* not recovered. Other organisms recovered.

TABLE V.

Eight animals injected with a 24-hour culture of *B. poncei* in nitrate solution. ♀ ♀ *M. femur-rubrum* used.

| No. of days..... | 1 | 2 | 3 | 4 | 5 | |
|---|---|---|---|---|---|---|
| No. of deaths.... | | | 2 | | 2 | 4 lived for a month |

TABLE VI.

Three animals injected with an emulsion of intestinal contents of last two dead in previous experiment. ♀ ♀ *M. femur-rubrum* used. 1st passage.

| No. of days..... | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| No. of deaths..... | | 2 | 1 | |

TABLE VII.

Three animals injected with an emulsion of intestinal contents of last one dead in previous experiment. ♀ ♀ *M. femur- rubrum* used. 2nd passage.

| | | | | |
|---|---|---|---|---|
| No. of days..... | 1 | 2 | 3 | 4 |
| No. of deaths..... | | 3 | | |

Three animals dead in last experiment "tested." *B. poncei* not recovered. Other organisms recovered.

TABLE VIII.

Five animals injected with a 24-hour culture of *B. poncei* in nitrate solution. ♀ ♀ *Encoptolophus sordidus* used.

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| No. of days..... | 1 | 2 | 3 | 4 | 5 | 6 | |
| No. of deaths..... | | 2 | 1 | | | 1 | 1 lived |

TABLE IX.

Five animals injected with an emulsion of intestinal contents of last one dead in previous experiment. ♀ ♀ *Encoptolophus sordidus* used. 1st passage.

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| No. of days.... | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| No. of deaths.. | | | 1 | | | 1 | 1 2 lived |

Animals dead on 6th and 7th days "tested." *B. poncei not* recovered. Other organisms recovered.

TABLE X.

Five animals injected with an emulsion of intestinal contents of last one dead in previous experiment. ♀ ♀ *Encoptolophus sordidus* used. 2nd passage.

| | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| No. of days..... | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 |
| No. of deaths..... | | | | | | | | 1 | | 1 | | 1 | | | | | 1 | | 1 |

Animals dead on 12th, 17th and 19th days "tested." *B. poncei* recovered from animal dead on 12th day. Other organisms recovered from remaining two tests.

TABLE XI.

Thirty-five animals injected with a 24-hour bouillon culture of *B. poncei*. ♀ ♀ *M. femur-rubrum* used.

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| No. of days.... | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
| No. of deaths.. | | 9 | 7 | 12 | 4 | 2 | | | | | 1 |

Three dead animals "tested" on 4th day as well as last seven dead. Recovered *B. poncei* three times. Other organisms recovered in remaining tests.

TABLE XII.

Nineteen animals injected with a 24-hour bouillon culture killed by sterilizing in autoclave. Sterility verified by inoculating various media. ♀ ♀ *M. femur-rubrum* used.

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| No. of days... | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 |
| No. of deaths | | 2 | | 3 | | | 9 | 1 | 1 | 1 | 1 | | | 1 |

Six animals "tested." *B. poncei* not recovered. Two tests showed cultural sterility; four showed presence of other organisms.

TABLE XIII.

Nine animals enclosed in a battery jar and food sprayed with a 48-hour culture of *B. poncei*. Proportions of sexes not noted, but ♂ ♂ and ♀ ♀ of *M. femur-rubrum* used.

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| No. of days..... | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 0 | 10 |
| No. of deaths..... | | | | | | 2 | 2 | | | 5 |

Five dead animals "tested." *B. poncei* not recovered. Other organisms recovered.

TABLE XIV.

Eight animals enclosed in a battery jar and food sprayed with a 48-hour culture of *B. poncei* in nitrate solution. Four ♂ ♂ and four ♀ ♀ *M. femur-rubrum* used.

| No. of days..... | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| No. of deaths... | | | 1 | | 2 | 1 | | | | 1 | | 1 | | | | | 1 | | | 1 |

Three dead animals "tested." *B. poncei* not recovered. Other organisms recovered.

TABLE XV.

Twenty animals injected with a 17-hour bouillon culture of *Coccobacillus acridiorum* "Souche Cham." ♀ ♀ *M. atlanis* used.

| No. of days..... | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| No. of deaths..... | | 17 | 3 | |

Last three dead "tested." Piece of femural muscle removed aseptically and dropped into culture tube. Pure culture of "Souche Cham" obtained.

TABLE XVI.

Two out of seventeen dead on 2nd day in previous experiment taken and femural muscle triturated in 10 c. c. sterile $H_2O$. Eight ♀ ♀ *M. atlanis* injected. 1st passage.

| No. of days.... | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|
| No. of deaths.. | | 5 | 3 | | | | | |

Tests made from blood and femural muscle of dead animals reacted positively for "Souche Cham." No other organisms found.

TABLE XVII (No. 1 and 2).*

Eight animals injected from one dead on 3rd day in previous experiment and eight injected from another dead the same day. Femural muscle triturated as above. ♀ ♀ *M. atlanis* used. 2nd passage.

No. 1.

| No. of days..... | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| No. of deaths..... | | 5 | 3 | | |

No. 2.

| No. of days..... | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| No. of deaths..... | | 4 | 4 | | |

Last three dead in No. 1 and two dead from No. 2 examined. "Souche Cham" recovered. No other organisms found.

TABLE XVIII.

Ten animals infected by spraying corn leaves with 24-hour bouillon culture of "Souche Cham." Five ♂ ♂ and five ♀ ♀ *M. atlanis* used.

| No. of days..... | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| No. of deaths..... | | 4 | 1 | 4 | 1 | |

Three of dead animals "tested." "Souche Cham" recovered from feces and from alimentary tract. Two other organisms recovered.

TABLE XIX.

Eight animals infected by spraying corn leaves with 24-hour bouillon culture of "Souche Cham." Four ♂ ♂ and four ♀ ♀ *M. atlanis* used.

| No. of days..... | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| No. of deaths..... | | 2 | 6 | |

Three animals "tested." "Souche Cham" recovered from alimentary tract. One other organism recovered.

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*Signifies separate jars in which grasshoppers were kept, so really two separate experiments, otherwise I might have incorporated the two experiments in one table.

TABLE XX.

Nineteen animals infected by spraying corn leaves with 24-hour bouillon culture of "Souche Cham." *M. bivittatus* used. Proportion of sexes not noted.

| No. of days.... 1 | 2 | 3 | 4 | 5 | 6 | 7 | |
|---|---|---|---|---|---|---|---|
| No. of deaths.. | 3 worm parasitism | 2 | 2 | 2 | 4 | 3 worm parasitism | Rest died of worm parasitism |

Two dead on 3rd day "tested." "Souche Cham" recovered from alimentary tract. Other organisms recovered.

TABLE XXI.

Eight animals infected by spraying corn leaves with a 24-hour bouillon culture of "Souche Cham." Four ♂ ♂ and four ♀ ♀ *M. bivittatus* used.

| No. of days..... 1 | 2 | 3 | 4 | 5 | 6 | |
|---|---|---|---|---|---|---|
| No. of deaths..... | | | 4 | 1 | 2 | 1 lived |

One dead on 5th day "tested." "Souche Cham" recovered in pure culture from alimentary tract.

TABLE XXII.

Eight animals infected by spraying corn leaves with a 24-hour bouillon culture of "Souche Cham." Four ♂ ♂ and four ♀ ♀ *M. femur-rubrum* used.

| No. of days.... 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|
| No. of deaths.. | | | 4 | 2 | 1 | 1 |

Two dead on 5th day "tested." "Souche Cham" recovered from alimentary tract. Other organisms recovered.

TABLE XXIII.

Ten animals injected with a 24-hour culture of "Souche Sidi." ♀ ♀ *M. atlanis* used.

| No. of days.... 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|
| No. of deaths.. | 1 | 2 | 3 | 2 | 2 | |

Two animals dead on 5th day "tested." "Souche Sidi" recovered from blood in pure culture.

TABLE XXIV.

Ten animals infected by spraying corn leaves with a 24-hour culture of "Souche Sidi." Five ♂ ♂ and five ♀ ♀ *M. atlanis* used.

| No. of days.... 1 | 2 | 3 | 4 | 5 | 6 | 7 | |
|---|---|---|---|---|---|---|---|
| No. of deaths.. | 2 worm parasitism | 1 | 1 | 2 | 1 | 1 | 2 lived for 10 days and then died naturally after depositing eggs. |

Two dead animals "tested." "Souche Sidi" recovered from alimentary tract. Other organisms recovered.

TABLE XXV.

Eight animals infected by spraying corn leaves with a 24-hour bouillon culture of "Souche Sidi." *M. bivitattus* used. Sexes not noted, but majority females.

| No. of days..... 1 | 2 | 3 | 4 | 5 | |
|---|---|---|---|---|---|
| No. of deaths.... | | 4 | 1 | 1 | 2 lived for about 10 days and then died after depositing eggs |

One animal dead on 4th day "tested." "Souche Sidi" recovered from alimentary tract. Other organisms recovered.

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